

IN THE CLAIMS

The status of each claim is provided below.

Claims 1-81: Canceled.

82. (New) A quantitative polymorphous analysis method, comprising:  
amplifying a target gene and monitoring the amplification by real-time PCR;  
performing a polymorphous analysis with respect to the amplified target gene to  
determine a polymorphous composition ratio of individual species of the target gene; and  
determining the initial amount of the target gene; and  
determining the initial amounts of individual species of the target gene.

83. (New) The method of Claim 82, wherein the real-time PCR is accomplished with  
a nucleic acid probe,

wherein the probe comprises a single-stranded oligonucleotide capable of hybridizing  
to the target nucleic gene,

wherein the probe is labeled with a fluorescent dye and a quencher substance,

wherein the oligonucleotide is labeled with the fluorescent dye and the quencher  
substance such that the intensity of fluorescence in a hybridization reaction system increases  
when the probe is hybridized with the target gene, and

wherein the oligonucleotide forms no stem-loop structure between bases at positions  
where the oligonucleotide is labeled with the fluorescent dye and the quencher substance.

84. (New) The method of Claim 82, wherein the real-time PCR is accomplished with  
a nucleic acid probe,

wherein the probe is labeled at an end portion thereof with a fluorescent dye, and  
the probe has a base sequence designed such that, when the probe hybridizes at the end portion thereof to the target nucleic acid, at least one G (guanine) base exists in a base sequence of the target gene at a position 1 to 3 bases apart from an end base of the target nucleic acid hybridized with the probe;

whereby the fluorescent dye is reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

85. (New) The method of Claim 82, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe is labeled at an end portion thereof with a fluorescent dye, and  
wherein the probe has a base sequence designed such that, when the probe hybridizes to the target gene, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at the end portion;

whereby the fluorescent dye is reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

86. (New) The method of Claim 82, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe is labeled with a fluorescent dye, wherein the probe is labeled at a position other than a 5' end phosphate group or a 3' end OH group thereof with the fluorescent dye, and

wherein the probe has a base sequence designed such that, when the probe hybridizes to the target gene, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at the modification portion; whereby the fluorescent dye is

reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

87. (New) The method of Claim 82, wherein the real-time PCR is accomplished with a nucleic acid probe, and determining an initial concentration of the amplified target gene from a percentage of a change in an intensity of fluorescence occurred as a result of hybridization between to the probe and the amplified target gene.

88. (New) The method of Claim 82, wherein the real-time PCR is accomplished with a nucleic acid probe as a primer, and including determining an initial concentration of the amplified target gene from a percentage of a change in an intensity of fluorescence occurred as a result of hybridization between the primer or an amplified nucleic acid amplified from the primer and the amplified target gene.

89. (New) The method of Claim 82, wherein the real-time PCR is accomplished with a nucleic acid probe, and including measuring an intensity of fluorescence in a reaction system in which the probe and the target gene or amplified nucleic acid have not hybridized with each other and also an intensity of fluorescence in the reaction system in which the probe and the target nucleic acid or amplified nucleic acid are hybridized with each other; and then calculating percentage of a decrease of the former intensity of fluorescence from the latter intensity of fluorescence.

90. (New) The method of Claim 83, further comprising correcting an intensity value of fluorescence in a reaction system, the intensity value being available after the target nucleic acid has hybridized to the nucleic acid probe labeled with the fluorescent dye, in

accordance with an intensity value of fluorescence in the reaction system available after a probe-nucleic acid hybrid complex so formed has been denatured.

91. (New) The method of Claim 83, further comprising, as a correction processing step, correcting an intensity value of fluorescence in a reaction system, the intensity being available in each cycle after the amplified nucleic acid has conjugated to the fluorescent dye or after the amplified nucleic acid has hybridized to the nucleic acid probe labeled with the fluorescent dye, in accordance with an intensity value of fluorescence in the reaction system available after a nucleic acid-fluorescent dye conjugate or probe-nucleic acid hybrid complex so formed has been denatured in the cycle.

92. (New) The method of Claim 91, wherein the correction-processing step is performed in accordance with the following formula (1) or formula (2):

$$f_n = f_{hyb, n} / f_{den, n} \quad (1)$$

$$f_n = f_{den, n} / f_{hyb, n} \quad (2)$$

wherein

$f_n$ : correction-processed value in an  $n$ th cycle as calculated in accordance with the formula (1) or formula (2),

$f_{hyb, n}$ : intensity value of fluorescence of the reaction system available after the amplified nucleic acid has conjugated to the fluorescent dye or the amplified nucleic acid has hybridized to the nucleic acid probe labeled with the fluorescent dye in the  $n$ th cycle, and

$f_{den, n}$ : intensity value of fluorescence of the reaction system available after the formed fluorescent dye-nucleic acid conjugate or the formed probe-nucleic acid hybrid complex has dissociated in the  $n$ th cycle.

93. (New) The method of Claim 92, further comprising:

introducing correction-processed values which have been calculated in accordance with the formula (1) or formula (2) in individual cycles, into the following formula (3) or (4) to calculate rates or percentages of changes in fluorescence between samples in the individual cycles:

$$F_n = f_n/f_a \quad (3)$$

$$F_n = f_a/f_n \quad (4)$$

wherein

$F_n$ : rate or percentage of a change in fluorescence in an  $n$ th cycle as calculated in accordance with the formula (3) or formula (4),

$F_n$ : correction-processed value calculated in the  $n$ th cycle as calculated in accordance with the formula (1) or formula (2), and

$f_a$ : correction-processed value calculated in a given cycle before a change in  $f_n$  is observed as calculated in accordance with the formula (1) or formula (2); and comparing the rates or percentages of changes in fluorescence.

94. (New) The method of Claim 92, further comprising:

1) performing processing in accordance with the following formula (5), (6) or (7) by using data of rates or percentages of changes in fluorescence as calculated in accordance with the formula (3) or (4):

$$\log_b(F_n), \ln(F_n) \quad (5)$$

$$\log_b\{(1-F_n) \times A\}, \ln\{(1-F_n) \times A\} \quad (6)$$

$$\log_b\{(F_n-1) \times A\}, \ln\{(F_n-1) \times A\} \quad (7)$$

wherein

$A, b$ : desired numerical values, and

$F_n$ : rate or percentage of a change in fluorescence in an  $n$ th cycle as calculated in accordance with the formula (3) or formula (4),

2) determining a cycle in which the processed value of the processing step 1) has reached a constant value,

3) calculating a relational expression between cycle of a nucleic acid sample of a known concentration and the number of copies of the target nucleic acid at the time of initiation of a reaction, and

4) determining the number of copies of the target nucleic acid in an unknown sample upon initiation of PCR.

95. (New) The method of Claim 82, wherein the polymorphous analysis is T-RFLP (terminal restriction fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSCP (single strand conformation) or CFLP (cleavage fragment length polymorphism).

96. (New) The method of Claim 82, wherein the polymorphous analysis is determined using a sequencer.

97. (New) The method of Claim 83, wherein the single-stranded oligonucleotide is labeled on the same nucleotide thereof with the fluorescent dye and the quencher substance.

98. (New) The method of Claim 83, wherein the distance between the bases at the positions where the oligonucleotide is labeled with the fluorescent dye and quencher substance, respectively, is 1 to 20 bases.

99. (New) The method of Claim 83, wherein the probe is labeled at a 3' end thereof with the fluorescent dye.

100. (New) The method of Claim 83, wherein the probe is labeled at a 5' end thereof with the fluorescent dye.

101. (New) The method of Claim 83, wherein the probe has G or C as a 3' end base and is labeled at the 3' end thereof with the fluorescent dye.

102. (New) The method of Claim 83, wherein the probe has G or C as a 5' end base and is labeled at the 5' end thereof with the fluorescent dye.

103. (New) The method of Claim 101, wherein a hydroxyl group on a 3' carbon of ribose or deoxyribose at the 3' end or a hydroxyl group on a 3' or 2' carbon of ribose at the 3' end has been phosphorylated.

104. (New) The method of Claim 83, wherein the probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group thereof with the fluorescent dye.

105. (New) The method of Claim 83, wherein the oligonucleotide of the probe is a chemically-modified nucleic acid.

106. (New) The method of Claim 105, wherein the chemically-modified nucleic acid is 2'-O-methyloligonucleotide, 2'-O-ethyloligonucleotide, 2'-O-butyloligonucleotide, 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

107. (New) The method of Claim 83, wherein the oligonucleotide of the probe is a chimeric oligonucleotide which comprises a ribonucleotide and a deoxyribonucleotide.

108. (New) The method of Claim 107, wherein the chimeric oligonucleotide comprises 2'-O-methyloligonucleotide, 2'-ethyloligonucleotide, 2'-O-butyloligonucleotide 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.



SUPPORT FOR THE AMENDMENTS

The specification has been amended to address the issues discussed by the Examiner at pages 6-7 of the Official Action. Newly-added Claims 82-108 are supported by the specification and the original claims. Accordingly, no new matter is believed to have been added to the present application by the amendments submitted above.

The specification has been amended to insert sequence identifiers where appropriate. Support for these amendments are found in the specification and sequence listing as originally filed. In addition, the Sequence Listing has been amended to add four sequences appearing in the specification as filed, but omitted from the original Sequence Listing. No new matter has been entered by these amendments.